RNase I (see the Office Action dated March 22, 2001 at page 3: "Meador et al. teach ... a method comprising culturing cells constitutively expressing the non-specific RNase, RNase I, lysing said cells and purifying the cellular component RNase I." (Citation omitted); page 4, "It would have been obvious to one of ordinary skill in the art at the time the invention was made to purify *E. coli* RNase I, as taught by Meador *et al.*..."; and page 6, "Meador et al. teach a method of purifying *E. coli* RNase I.").

However, Applicants note that in the first Office Action on the merits, dated October 3, 2000, the Examiner rejected claims under 35 U.S.C. 112 as "incomplete for omitting essential steps" noting that the omitted steps was "isolating said RNA-free cellular component" (see Office Action dated October 3, 2000, page 4).

The Examiner is reminded that according to the MPEP706.07(a):

> A second or any subsequent office action on the merits ... should not be made final if it includes a rejection, on prior art not of record, of any claim amended to include limitations which should reasonably have been expected to be claimed. See MPEP 904 *et seq.* For example, one would reasonably expect that a rejection under 35 U.S.C. 112 for the reason of incompleteness would be replied to by an amendment supplying the omitted element.

As the Applicants' amended the claims to supply the omitted element, the Examiner reasonably should have expected this element to be claimed. Thus, the Action should not have been made final. We urge the Examiner to reconsider, and withdraw the finality of the rejection.

Applicants have amended claims 1, 3, and 36 to clarify the distinction between the dependent claims 3 and 36. Claim 1 does not specify that the cells which produce the cellular component also produce the RNase. It is only specified that the cell lysate contains both the cellular component and the RNAse. Claim 3 is drawn to an embodiment in which the cells producing the cellular component and the cells producing RNase are different cells. Claim 36 is drawn to an embodiment in which the same cells that produce the cellular component also produce the RNase. We respectfully request withdrawal of the rejection under 37 C.F.R. § 1.75(c).

Turning to the merits of the application, the claims are clearly patentable over the teachings of Meador *et al.* (1989) *Eur. J. Biochem.* 187:549-553 ("Meador"). Meador teaches the expression of RNase in the periplasm of bacteria and subsequent purification of RNase from the periplasm while *minimizing cell lysis.* As described in Meador, page 549, column 2 under "Enzyme purification": "RNase I was purified from freshly grown bacteria, since some lysis occurred upon freeze-thawing to release DNA and other components that made subsequent purification difficult." As described in the same paragraph of Meador, cell lysis made subsequent purification of RNAse I difficult. The periplasm, or periplasmic space, is the region between the inner and outer membranes of a Gram-negative bacterial cell. Spheroplasts are formed when the outer membrane of the Gram-negative bacterium is partially removed. Thus, purification of enzymes from the periplasm by the formation of spheroplasts does not involve lysis of cells. Contrary to the Examiner's assertion on page 3, Meador does not disclose a "cellular lysate [that] comprises cellular components and RNase with sufficient activity to degrade all the RNA molecules present in the cell lysate." Rather, Meador took considerable pains to avoid cell lysis and to ensure that the RNase did not become contaminated with cellular components (including RNA).

In sharp contrast to the teachings of Meador, an aspect of the Applicants invention is that, at the appropriate time, the cells are lysed to allow the periplasmic (or otherwise extracellular) RNase to access its intended intracellular RNA substrate. As Meador purified RNase from spheroplasts, Meador did not perform, teach or suggest that cellular components could be purified by lysis of cells containing cellular components and RNase such that the RNase could degrade substantially all of the RNA in the cell lysate. Meadow simply did not make a cell lysate. We respectfully request withdrawal of the rejection of claims under 35 U.S.C. § 102(b).

The Examiner asserts that it would have been obvious for one of ordinary skill in the art to overexpress RNase I, as taught by Zhu *et al.* (1990) *J. Bacteriol.* 172(6):3146-3151 ("Zhu"), using the method of Meador. However, Zhu merely identified the gene for RNase I by identifying cells with increased RNase activity. Zhu expressed RNase in RNase-deficient *E. coli* cells transfected with cosmids from a cosmid library. Zhu's goal was to identify RNase genes, not to isolate RNase-

free cellular components. As such, Zhu was only reconstituting a defective *E. coli* strain back to essentially a wild-type. Moreover, the method taught by Meador specifically teaches away from lysing cells. Meador notes that cell lysis made purification of RNase I difficult. Therefore, one of skill in the art would not have a reasonable expectation of success that the method of Meador would allow one to purify RNase or any other cellular component from a cell lysate. Thus, one of ordinary skill in the art would not arrive at the invention without contradicting the specific teachings of Meador.

Moreover, contrary to the Examiner's assertion, Zhu does not disclose RNase with sufficient activity to degrade all the RNA molecules present in the cell lysate. Both Zhu and Meador assay RNase activity by means of *exogenously added*, radiolabeled RNA substrates. There is no assertion or indication in either reference that there is sufficient RNase activity to degrade all RNA molecules present in the cell. In fact, such cellular RNA degradation does not occur, as evidenced by the viability of the cells, which could not function without their cytoplasmic RNA.

We respectfully request withdrawal of the rejection of claims 7, 8, and 12 under 35 U.S.C. § 103(a) over Meador in view of Zhu.

The Examiner also alleges that claims 13 and 17 are obvious over Meador in view of Zhu and further in view of Clare *et al.* (1991) *Gene* 105:205-212 ("Clare"). Clare is cited for teaching inducible production of a protein (mouse epidermal growth factor) in yeast. The Examiner argues that the teachings of Clare are general for the inducible production of protein and it would have been obvious to "lyse the cells for the benefits of secretion production of RNase I...." However, as noted above, Meador specifically teaches away from lysing cells as the cellular components released upon cell lysis made the purification steps difficult. Further, in Clare's system, the goal is to obtain the recombinant product in the medium in which the cells are grown, and from which the cells may be easily separated without having to lyse them. It would be contrary to the teaching of using secretion vectors to, nevertheless, lyse the cells.

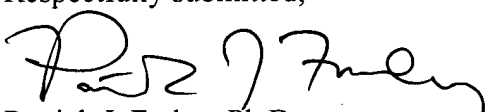
Moreover, purification of RNase from spheroplasts is unique to Gram negative bacterial cells and such a method is inapplicable to yeast cells. Thus, the combination of Clare, Zhu and Meador fails to achieve the invention as claimed.

Finally, the Examiner asserts, "Applicants have not furnished evidence that a bacterial cell expressing RNase I would not have sufficient RNase activity to degrade substantially all the RNA molecules present in the cell lysate." However, it is well-known that bacterial cell lysates contain contaminating RNA despite expression of various RNases, evidenced by a characteristic smear when plasmid preparations are run on agarose gels.

We respectfully request withdrawal of the rejection of claims 13 and 17 under 35 U.S.C. § 103(a) over Meador in view of Zhu and further in view of Clare.

In view of the foregoing, we request reconsideration, and urge prompt allowance of all the claims as amended.

Respectfully submitted,



Patrick J. Farley, Ph.D.
Registration No. 42,524

Date: June 21, 2001

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

Appendix A

1. (Twice Amended) A method of preparing a substantially RNA-free cellular component, comprising culturing cells [producing the cellular component] in a medium, wherein said cells comprise cellular component-producing cells, and lysing said cells to produce a cell lysate, wherein said cell lysate contains said cellular component and RNase with sufficient RNase activity to degrade substantially all of the RNA molecules present in said cell lysate, and isolating said cellular component.

3. (Twice Amended) The method of claim 1, wherein said cells further comprise RNase-producing cells [and cells comprising said cellular component] wherein the RNase is produced by said RNase-producing cells.

6. (Twice Amended) [The method of claim 4,] A method of preparing a substantially RNA-free cellular component, comprising culturing cells producing a cellular component and cells producing an RNase, wherein the cellular component and the RNase are not produced by the same cells, lysing said cells to produce a cell lysate, wherein said cells producing an RNase produce RNase in an amount sufficient to degrade substantially all of the RNA present in said cell lysate, and isolating said cellular component.

36. (Amended) The method of claim 1 wherein said cellular component-producing cells [producing said cellular component] produce said RNase.